New tools for control of bovine tuberculosis in cattle

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Bovine tuberculosis (TB) in New Zealand

Total cost of $100 million/year ($81 million TBfree NZ)

Possum control ($55 million, TBfree NZ)

Testing cattle and deer, compensation for reactors

At 30 June 2014, 69 cattle and 3 deer TB-infected herds

annual herd period prevalence of 0.21%

TB is endemic in wildlife in 39% of our country

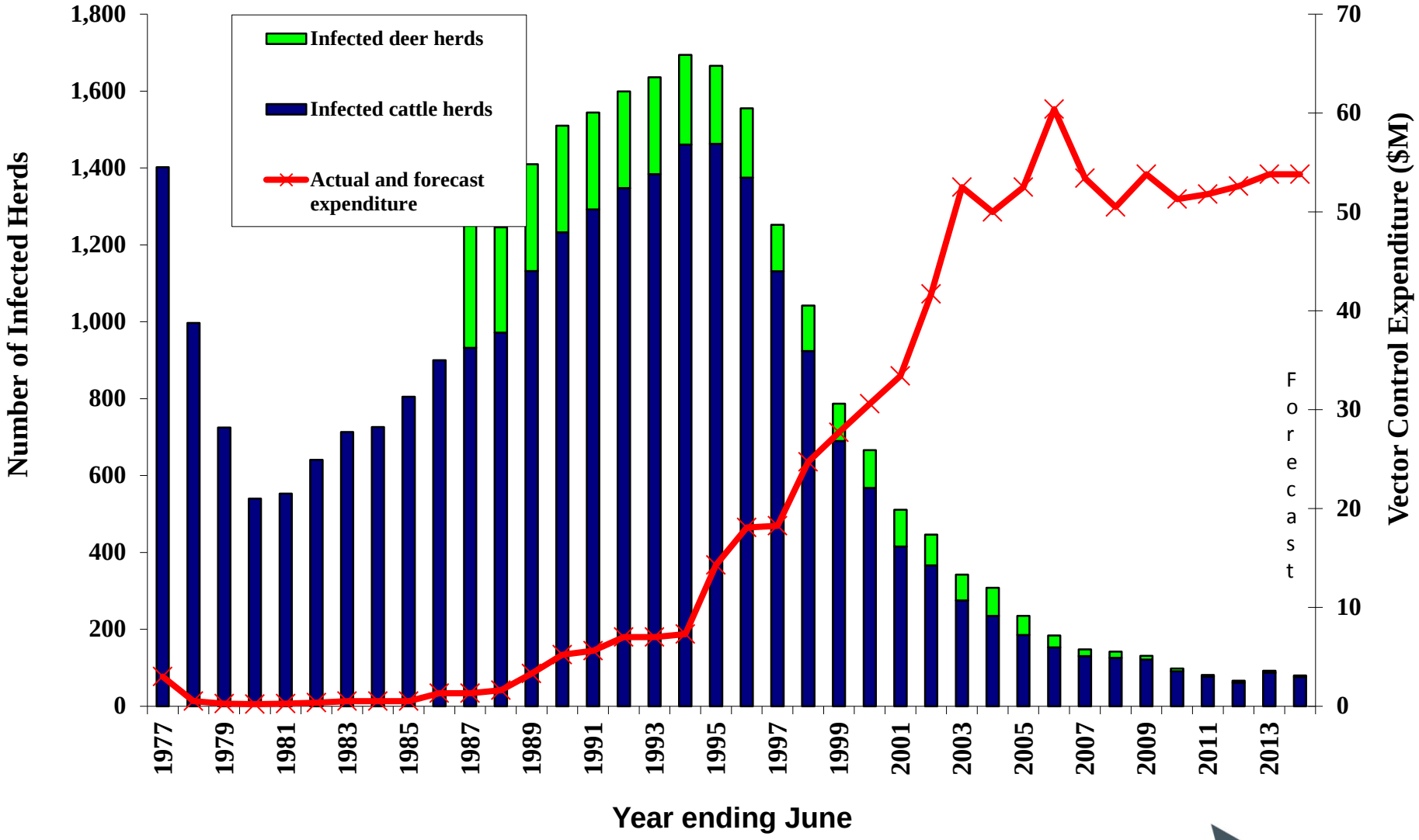
Need to reduce costs for cattle and deer TB control

- increase emphasise where TB endemic in wildlife
- funding could reduce – regional distribution of TB

Overview

- Progress in control of bovine TB in NZ
- Vaccines
  - Human TB vaccines
  - TB vaccines for cattle
    - Effect of revaccination
    - BCG field trials
- Improved diagnostic test – novel skin test reagent

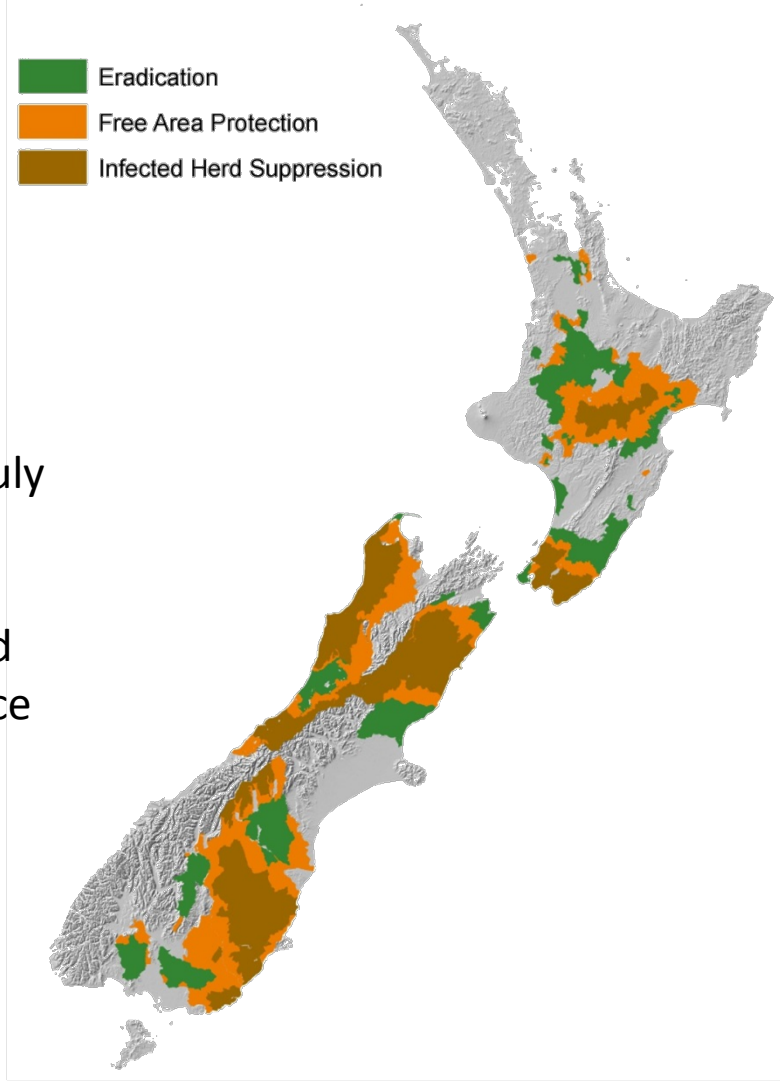
Number of infected cattle and deer herds and expenditure on vector control 1977 - 2014



National Bovine TB Pest Management Strategy 2011

Strategic choices

- 25% reduction in area where TB is endemic in wildlife by July 2026 (2.5 million hectares)
- Maintain national TB-infected annual herd period prevalence < 0.4%



Human BCG vaccination

Meta-analysis 86% efficacy against miliary and meningeal TB

Heterologous protection against pulmonary TB
(Northern hemisphere v tropics)

Protection wanes (7 of 10 trials), 14% efficacy after 10 yrs

Protection the same for different BCG strains

WHO – no benefit from revaccination with BCG

New TB vaccines for humans

Live mycobacterial vaccines to replace BCG

Attenuated *M. tuberculosis* strains

Recombinant BCG

- Over-expressing *M. tuberculosis* antigens
- Expressing listeriolysin or
- Enhance apoptosis

New TB vaccines (continued)

Subunit vaccines to enhance BCG

Virus-vectored vaccines

- Modified vaccinia virus Ankara (MVA – Ag85A)
- Non-replicating adenovirus type 35 (Ad-85A, 85B, TB10.4)

Protein vaccines

- 72F protein in ASO2
- Hybrid 1, 85B + ESAT-6 with IC31 adjuvant
- HyVac, TB10.4 + 85B with IC31 or CAF01

BCG vaccination of cattle

Advantages

- Inexpensive (low dose can be used)
- Safe
- DIVA tests to differentiate from *M. bovis* infection

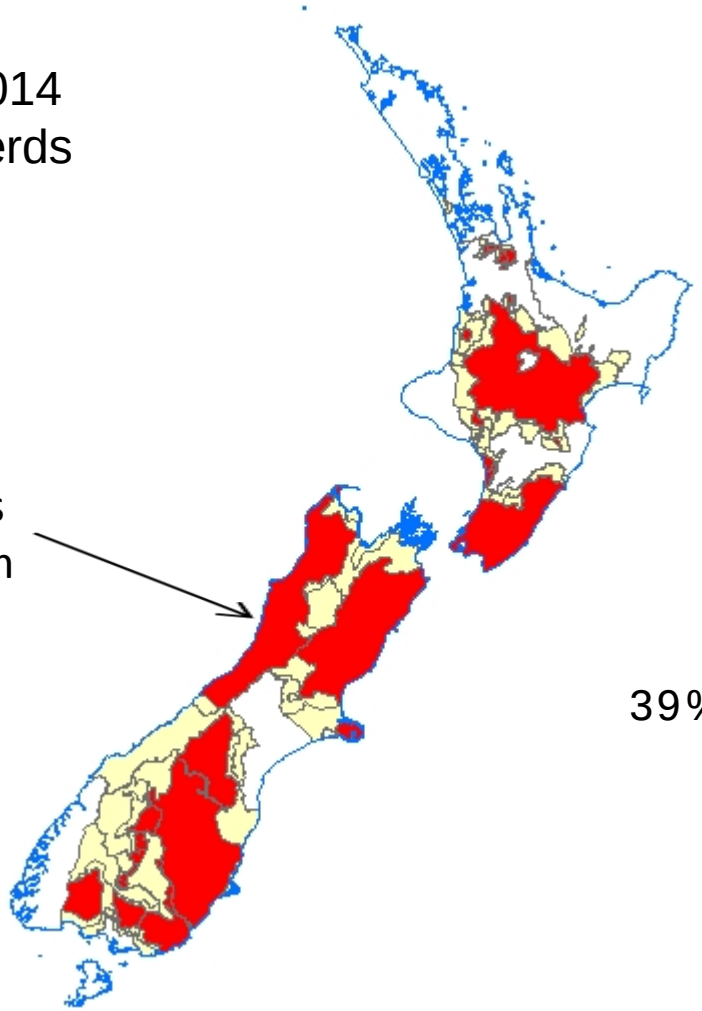
Disadvantages

- Proportion of vaccinated animals react in skin test
- Protection may be incomplete
- No therapeutic effect

Use of a TB vaccine for cattle

TB status – 30 June 2014
69 cattle and 3 deer herds infected

<u>West Coast</u>
2% cattle herds
55% TB-infected herds
$10 million for possum control



Location of
TB wild animals

Fringe
Endemic TB

39% of NZ has TB in wildlife

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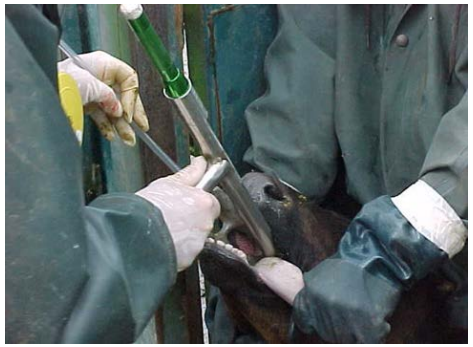
Endobronchial TB challenge of cattle

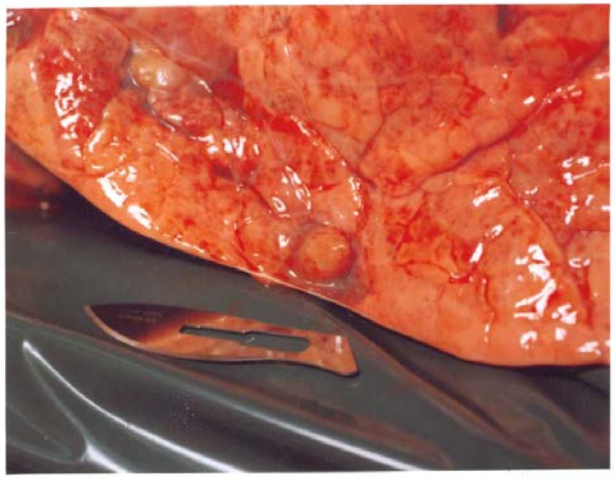
- Challenged with *M. bovis*



TB containment facility at Kaitoke

- Necropsy at 16 weeks after challenge

TB lesions



Summary: BCG vaccination of cattle

• Dose (10^3 to 10^6 CFU)	similar protection
• Strain of BCG / lyophilised v fresh culture	similar protection
• Age of animal	very young
• Pre-exposure to environmental mycobacteria	+ve or-ve
• Oral immunisation	effective
• Oral + systemic immunisation	no better
• Prime with BCG + boost with TB protein, DNA or virus-vector sub-unit vaccine	better than BCG alone
• Duration of immunity	12-24 mths

Effect of BCG revaccination in young calves

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Calf vaccine groups (n=10)

- Not vaccinated
- BCG within 8 hours of birth
- BCG at 6 weeks old
- BCG 8 hours + 6 weeks

Challenge with *M. bovis* at 14-17 weeks, necropsy 4 months later

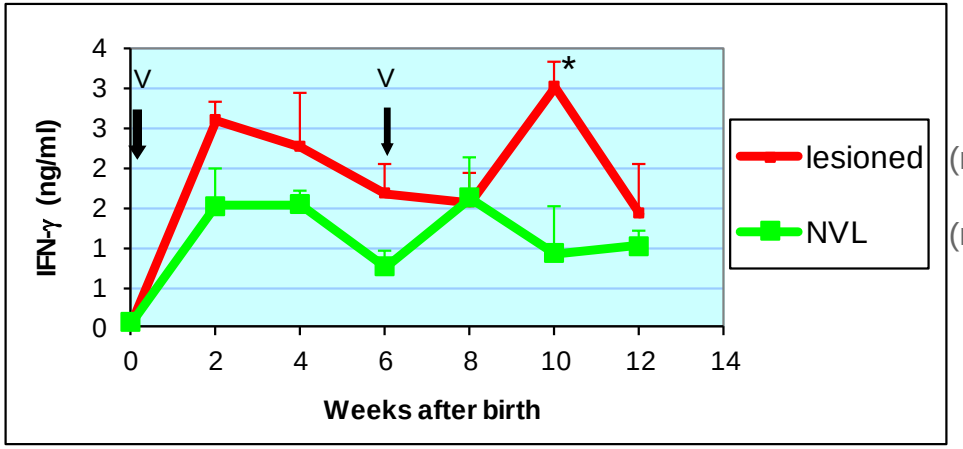
Proportion with TB lesions

• Not vaccinated	10/10
• BCG within 8 hours of birth	0/10
• BCG at 6 weeks old	1/9
• BCG 8 hours + 6 weeks	4/10

Ref. Buddle et al., Infect Immun. 2003

Immune responses of calves re-vaccinated with BCG

IFN-γ



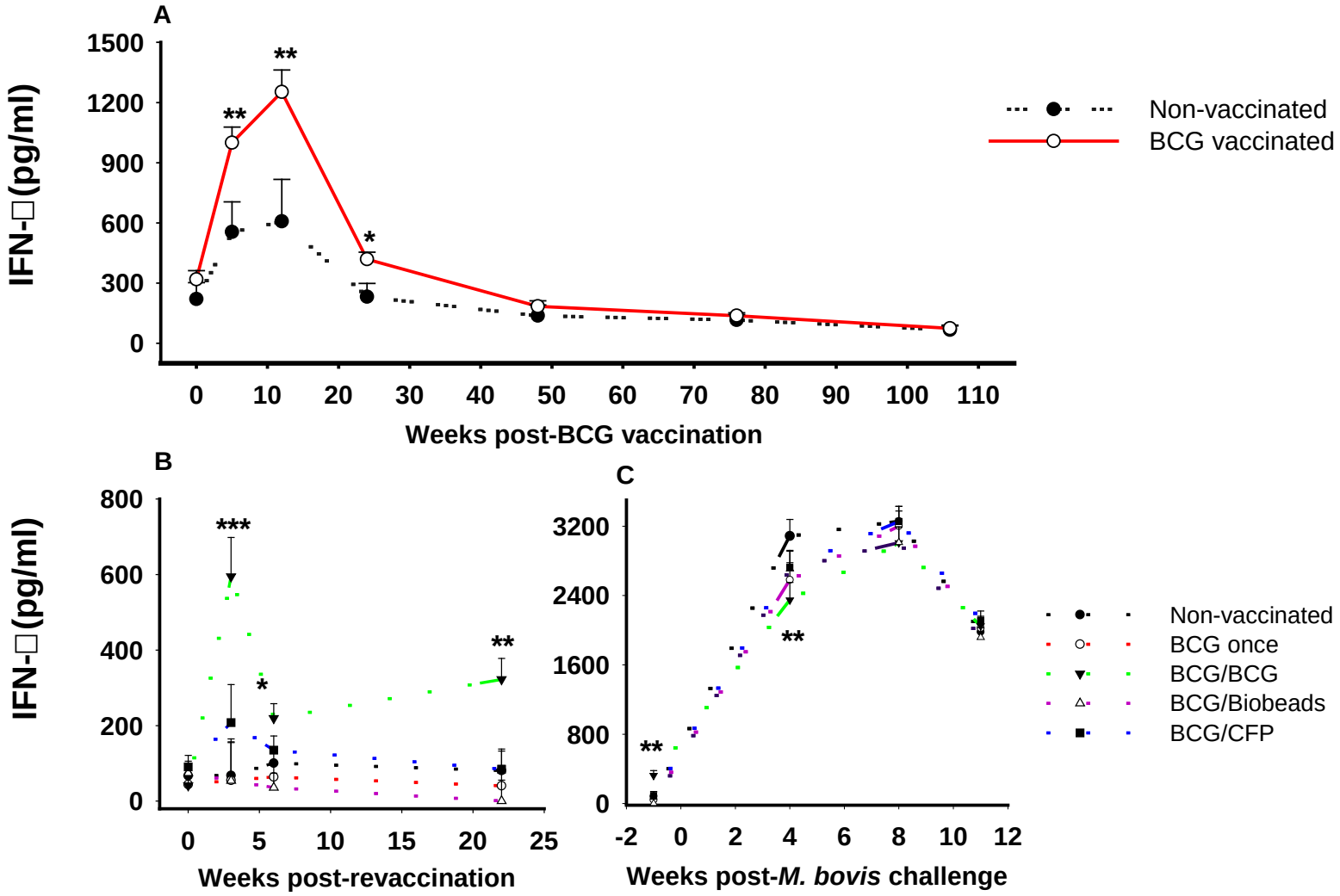
Animals which subsequently developed lesions had the highest post-vaccination immune responses

Long term effects of BCG vaccination and can immunity be boosted?

Vaccine groups (total 79 calves)

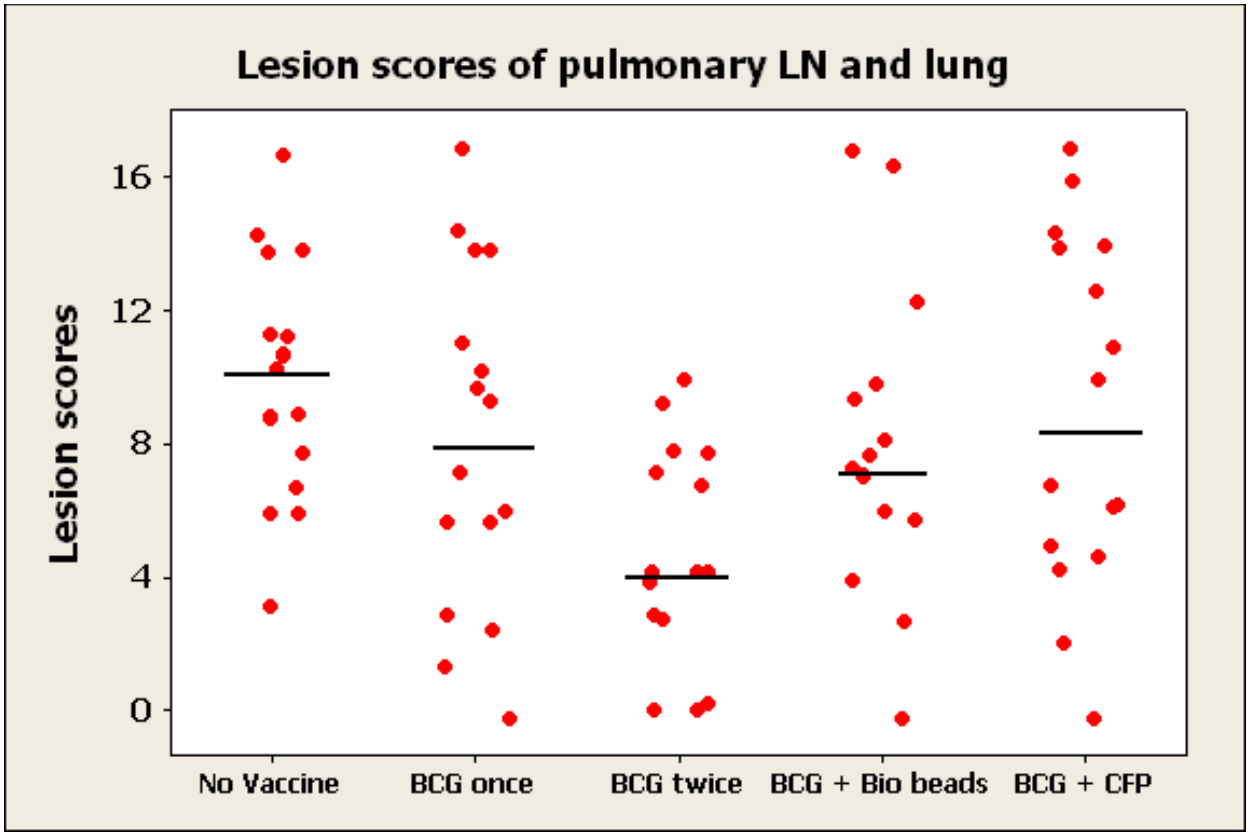
1. Non-vaccinated (n=17)
2. S/c BCG (n=16)
3. S/c BCG, at 2 years revaccinate BCG (n=15)
4. S/c BCG, at 2 years revaccinate with *M. bovis* culture filtrate protein (CFP)/adjuvant (n=15)
5. S/c BCG, at 2 years revaccinate with Biobeads displaying mycobacterial proteins, ESAT-6 and Ag85A on the surface (n=16)

Whole blood IFN-γ responses to bovine PPD



Significant different to the non-vaccinated group, * P <0.05, ** P <0.01, *** P<0.001

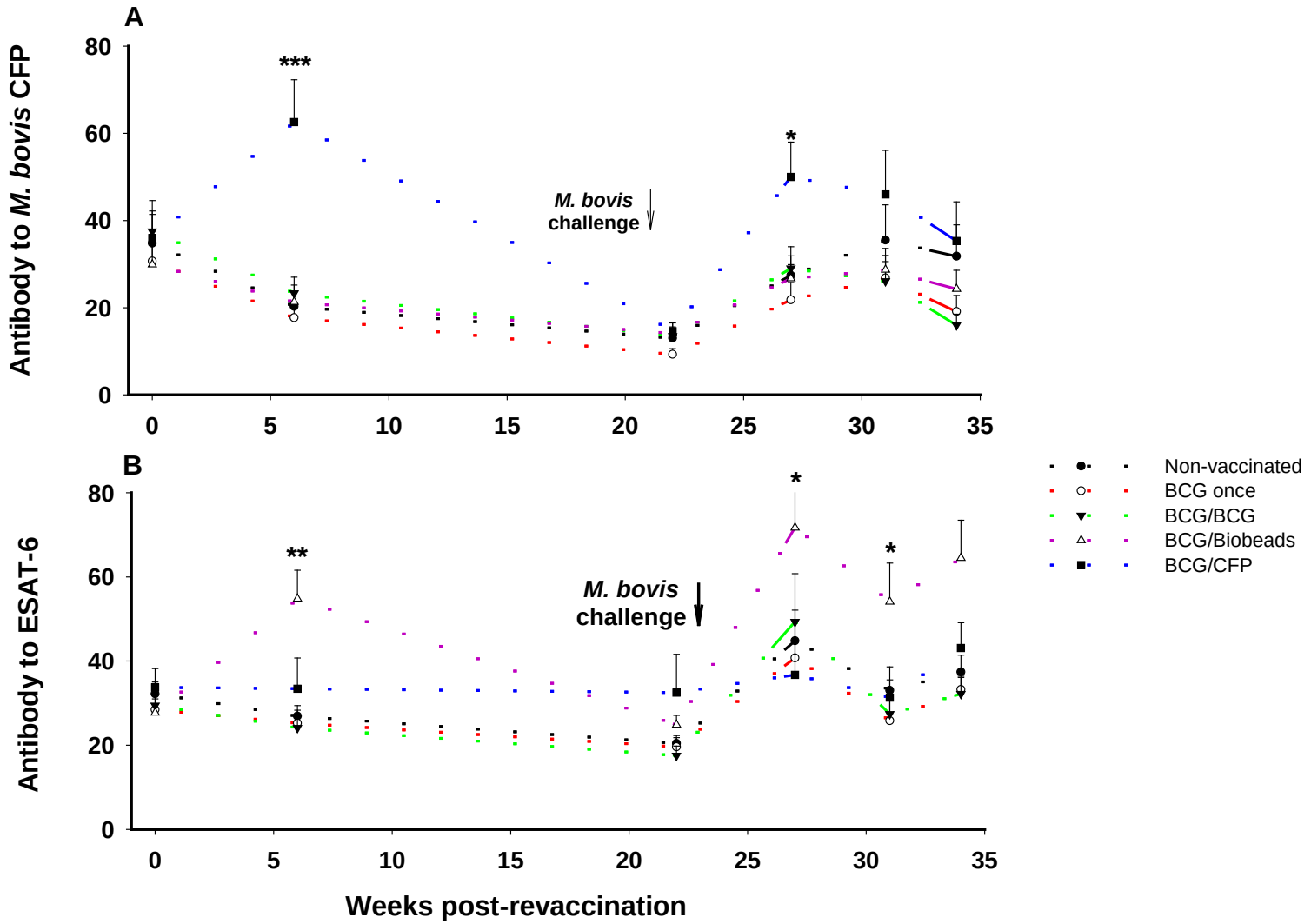
Total lung and pulmonary lymph node lesion scores following challenge with *M. bovis*



BCG-revaccinated group had significantly lower lesion scores than no vaccine group ($P < 0.001$)

Parlane et al., PLOS ONE, 2014

Serum antibody responses to *M. bovis* CFP and ESAT-6



Significant different to the non-vaccinated group, * P<0.05, **P<0.01, ***P<0.001

Cattle vaccination trial:

Muzzle Station (LandcareResearch, NZ)

- Isolated farm
- TB incidence 5 -10% of cattle/yr

BCG vaccination trial

- Five cohorts of ‘free ranging’ cattle, skin-tested, positives excluded.
- Approx. half vaccinated with BCG orally (mostly 10^8 CFU)
- Cattle inspected for TB at slaughter 1-3 yr later.

Progress results

Provisional diagnoses, some cultures pending

Close to final sample sizes except for 2010 cohort

Cohort birth year	Oral BCG Dose	Vaccinates	Non Vaccinates	*P* 2 x2 contingency table
2.5 yr cattle 2006	10^8	0/30 (0.00%)	5/130 (3.85%)	0.58
1.5 yr cattle 2007	10^8	5/172 (2.91%)	8/118 (6.78%)	0.15
Weaners 2008	10^8	11/177 (6.21%)	12/85 (14.12%)	0.039
Weaners 2009	10^8	10/167 (5.88%)	21/106 (19.81%)	<0.001***
Weaners 2010	2×10^7	2/98 (2.04%)	7/84 (8.33%)	0.083
Total		28/644 (4.35%)	53/523 (10.13%)	<0.001***

Diagnostic tests to enhance specificity

Differentiate Infected from Vaccinated Animals (DIVA tests)

- Whole blood IFN-γ test (ESAT-6, CFP10, Rv3615c)
- Differential skin test (ESAT-6, CFP10, Rv3615c)

Primary screen in UK - comparative cervical skin test (avian and bovine PPD)

Primary screen in NZ- caudal fold skin test (bovine PPD)

5 million cattle tested/year

Diagnostic tests to enhance specificity

Differentiate Infected from Vaccinated Animals (DIVA tests)

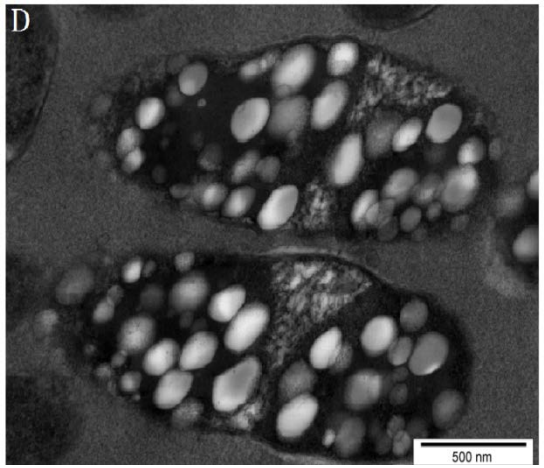
- Differential skin test (ESAT-6, CFP10, Rv3615c)
  - Recombinant proteins or peptides (DEFRA, UK)
  - Proteins displayed on biobeads (AgResearch/Massey)
    - Antigens presented on particles are more immunogenic
    - Reduce reagent cost
      - Use low antigen concentration (30-fold reduction)
      - Fermentation technology

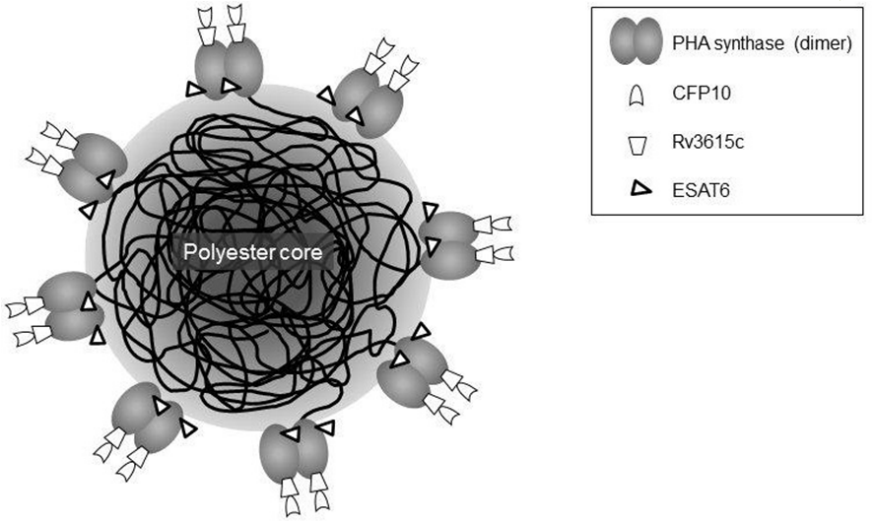
Biobeads displaying mycobacterial proteins

Under certain nutritional conditions, bacteria can produce polyester beads (Biobeads)

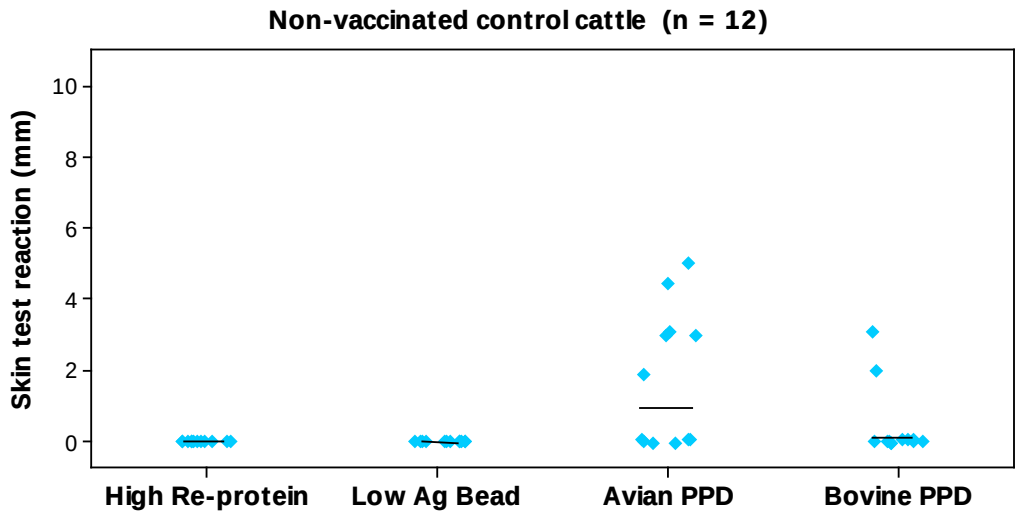
Foreign antigens can be displayed on these beads by translationally fusing them to polyhydroxyalkanoate (PHA) synthase which mediates the bead formation in recombinant *Escherichia coli.*

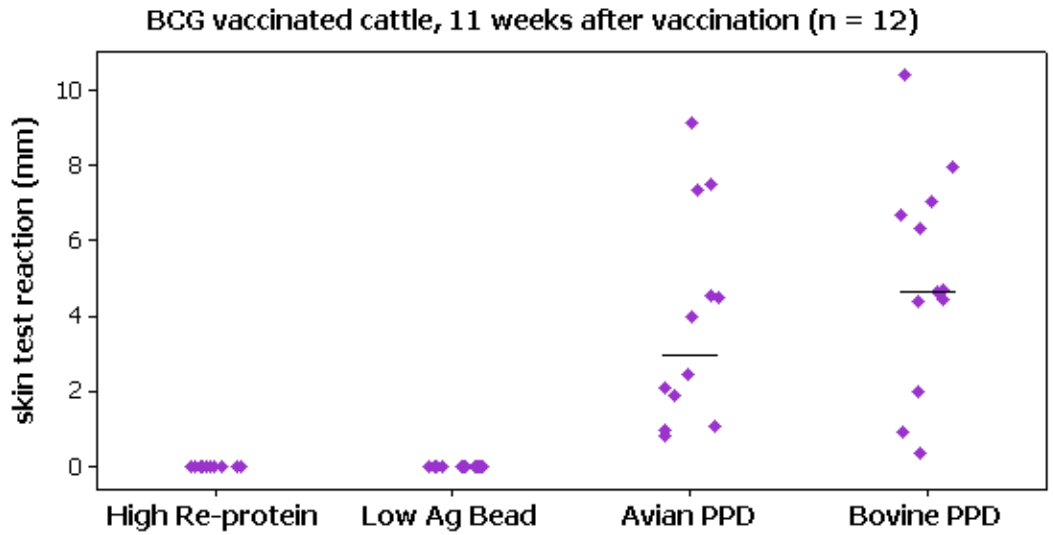
Beads are purified and antigens are recognised by SDS-PAGE, MALDI-TOF mass spectrometry and ELISA



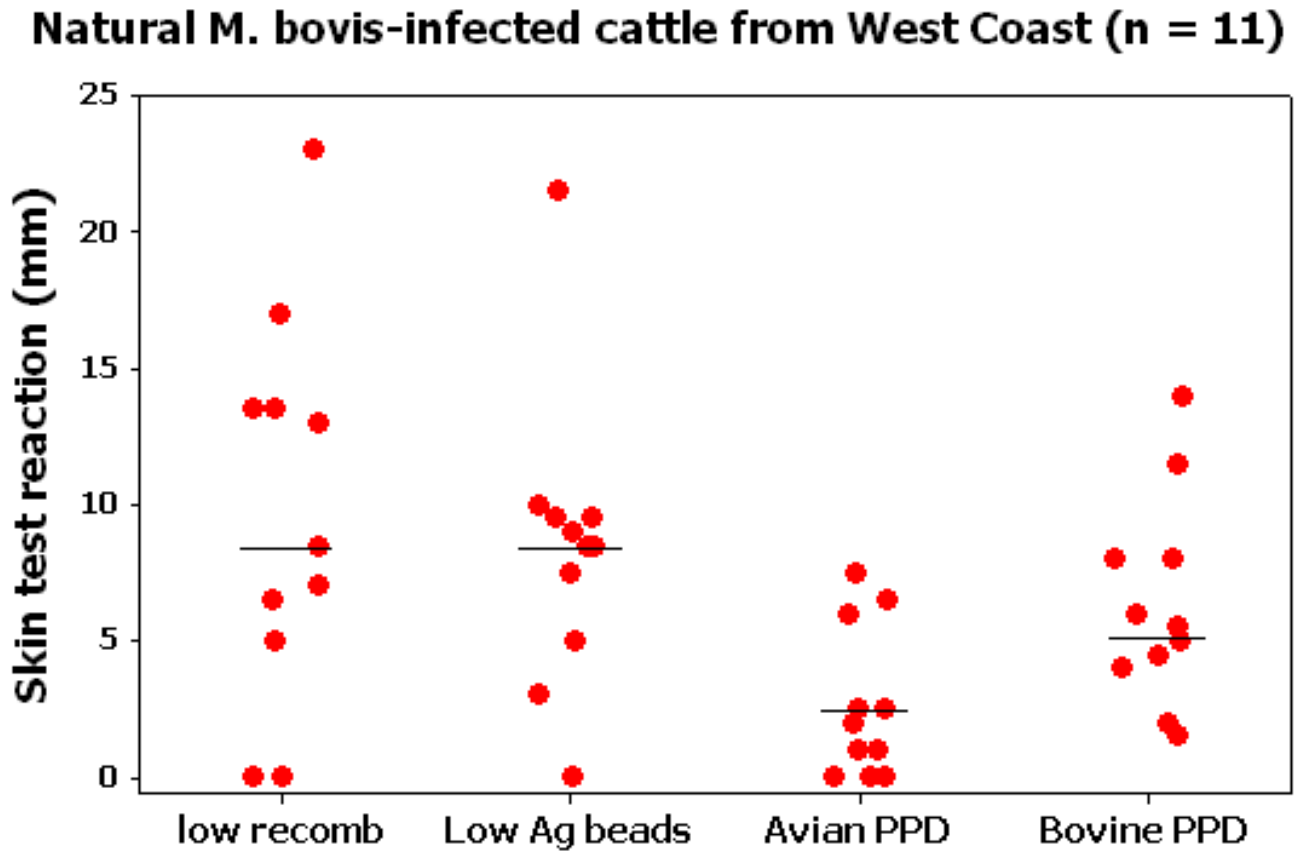


Skin test responses of non-vaccinated and BCG-vaccinated cattle





Skin test responses in cattle naturally-infected with *M. bovis*



3 Protein Biobeads have positively identified 41 of 42 experimentally or naturally *M. bovis*-infected cattle

Further developments with Biobead skin test reagent in cattle

- Added a fourth mycobacterial protein to the biobeads to enhance test sensitivity
- Reduced sedimentation of beads (resuspended in 15% dextran)
- Sterilised beads by gamma-irradiation
- Tested for sensitisation using multiple doses in guinea pigs
- Tested the 4 protein Biobead reagent in caudal fold test (9/9 positives)
- Commenced a large field trial (25,000 to 30,000 animals) to obtain accurate estimates of sensitivity and specificity using the comparative caudal fold test (4-protein Biobead v. bovine tuberculin)

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Questions?